THE AIR TURBINE FOR HIGH SPEED CENTRIFUGING OF BIOLOGICAL MATERIAL, TOGETHER WITH SOME OBSERVATIONS ON CENTRIFUGED EGGS

E. NEWTON HARVEY

(From the Loomis Laboratory, Tuxedo Park, New York, the Physiological Laboratory, Princeton University, and the Marine Biological Laboratory, Woods Hole)

There are two main difficulties to be encountered in centrifuging living cells at high speed. First, they may be crushed against the bottom of the container by the high forces developed. This can be obviated by suspending the material in a medium of graded density so that it comes to lie in a stratum of equal density and is completely cushioned against crushing. For this purpose mixtures of isotonic sucrose or raffinose [1] and salt solution may be used with material normally bathed in sea water or salt solution, while neutralized gum arabic will serve for fresh water forms.

Second, heating of material is bound to occur due to air friction unless the centrifuge is run in a vacuum or in hydrogen at low pressure, a procedure which introduces complicated accessory apparatus and is decidedly objectionable from the biological standpoint.

This difficulty is avoided by the use of the air turbine (Henriot and Huguenard 1925, 1927) perfected by Beams (1930, 1931, 1933) and fully described by him. Suffice it to say that a small top-shaped bearingless rotor (Fig. 1) with thirty grooves or flutings cut in its sides revolves on an air cushion of whirling jets of air, which hold it in place by the principle of Bernouilli. The constantly expanding air maintains the rotor a few degrees below room temperature. There are eight jets of air in the stator [2] I have been using, coming from diagonally bored holes of $\frac{1}{32}$ inch diameter which connect with a reservoir of air at a pressure that can be varied from 0 to 120 lbs. per sq. in. (Figs. 2 and 3). A vertical hole of $\frac{1}{12}$ inch diameter in the bottom of the stator is connected with an air supply at lower pressure and serves as a supporting jet. By the adjustment of the rotating air pressure and the supporting air pressure, rotors of varying size and shape can be made to revolve smoothly. A more recent design (Beams, Weed, and Pickels, 1933) of the stator omits the supporting pressure. The vertical hole is open to the atmosphere and the air sucked in automatically maintains the rotor

[1] A trisaccharide with higher molecular weight and greater density for isosmotic concentrations.

[2] Kindly constructed for me by Mr. A. J. Weed under Dr. Beams' direction.

in a stable position of rotation. Garman (1933) has described details of construction for efficient rotation.

Since eight holes of $\frac{1}{32}$ inch will deliver 1.61 cu. ft. of air at 100 lbs. pressure and one hole of $\frac{1}{12}$ inch diameter will deliver 3 cu. ft. of air at 10 lbs. pressure, the total air capacity necessary is about 18.7 cu. ft. per minute at 100 lbs. pressure. The air compressor should be capable of delivering this and should be a 5 H.P. outfit.

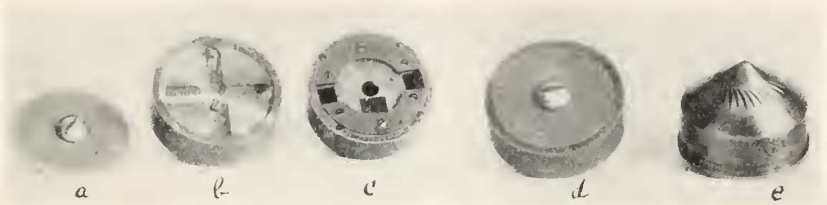

FIG. 1. Rotors for air turbine. (*a*) cover for tube type; (*b*) tube type rotor showing grooves for centrifuge tubes; (*c*) rotor for microscope observation; (*d*) tube type rotor with cover on; (*e*) bottom of rotor showing flutings.

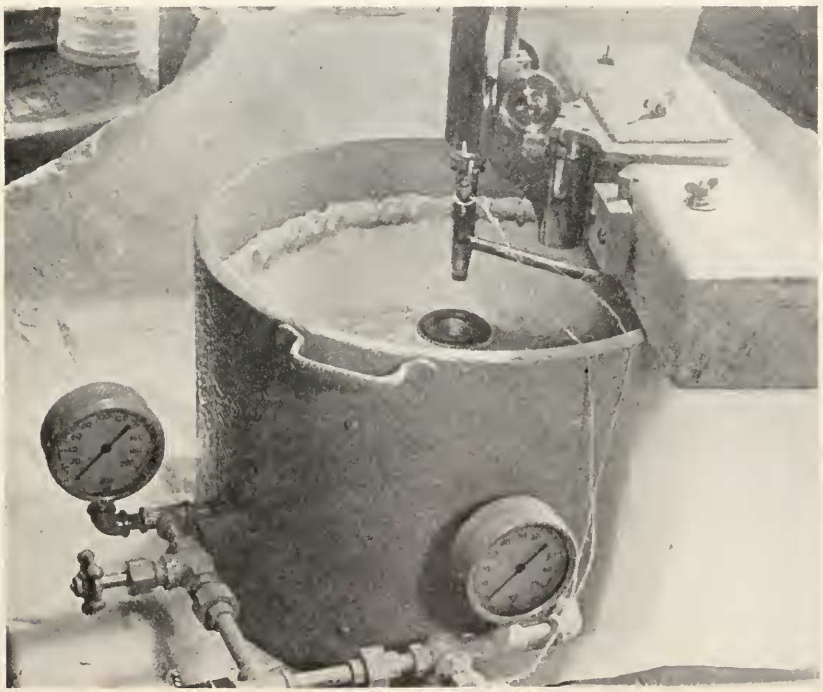

FIG. 2. Stator mounted in a steel protecting case with pressure gauges to read rotating and supporting pressures and microscope mounted for observing material. Note canvas for catching rotor when it is to be stopped or if it jumps out of stator.

The centrifugal forces developed at high pressures are enormous and adequate protection in the form of steel casing or sand-filled barriers must be provided to prevent injury from exploding rotors. A strip of canvas under the stator (Fig. 2) serves to catch the rotor if it becomes unstable and moves out of the stator.

Rotors of various types can be used. Beams (1931) has described rotors into which material can be introduced and from which it can be taken while rotating and also methods of observing the sedimentation velocity of particles (Beams, Weed, and Pickels, 1933). I have already described (Harvey, 1932) a type [2] designed for observation of material with the microscope while being centrifuged. The complete

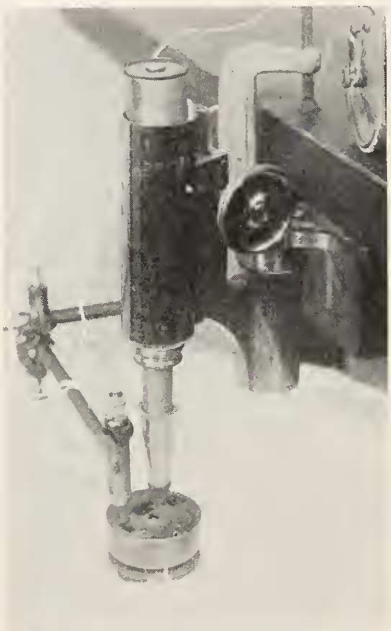

FIG. 3. Near view of rotor on stator with microscope and illumination.

outfit is shown in Fig. 3 with rotor resting on stator, microscope, and illumination. The optical system is shown in Fig. 4. The light is a small 2-volt electric flashlight bulb, an image of whose straight filament is thrown by a lens on the cells to be observed, parallel to a radius of rotation. The chamber to hold the cells is made from Pyrex tubing, sealed and flattened at one end, and ground and polished to fit in position in the rotor. The straight end of the chamber where the cells are thrown by centrifugal force must come in position over the stellite mirror so that its image is reflected into the tube of the microscope. A perfect

image is obtained. Careful construction of the chamber and a cushion of picene will prevent breakage at relatively high centrifugal forces, but special treatment of the glass (Beams, Weed, and Pickels, 1933) is necessary at very high speeds. The chamber is shown in Fig. 5, *A*.

For centrifuging in tubes the rotor[3] shown in Fig. 1 is most convenient. This rotor can be made of solid steel rod $1\frac{3}{4}$ inch diameter, turned to shape, with two crossed grooves to hold centrifuge tubes milled in the upper surface. A cover of duraluminum fits over the grooves and is held in place by a machine screw. For low speeds the tubes can be made of thick-walled Pyrex tubing, but for high speed they are best made of duraluminum or aluminum, which are light and do not easily corrode. Holes are bored in square duraluminum rod

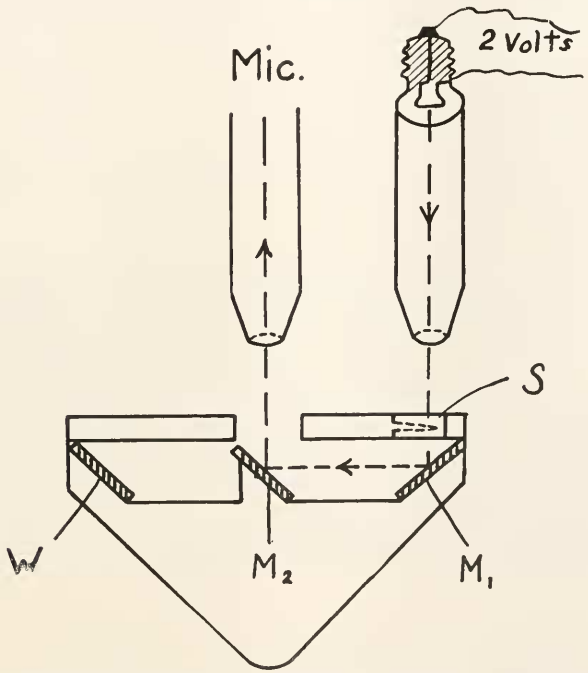

FIG. 4. Optical system for microscope observation of material rotating at high speed.

and the end rounded in one plane to fit the groove in the rotor as shown in Fig. 5, *B*. Only ordinary precautions are necessary to make tubes of the same weight, since the rotor assumes its own position of equilibrium when revolving and a considerable amount of unbalance does not interfere with the stability of the rotor, provided the supporting

[3] Constructed by Mr. P. Miller at the Loomis Laboratory, Tuxedo Park.

and rotating pressures are properly adjusted. The rotor is started by placing it on the stator and turning on the air. It is stopped by scraping it off the stator onto the canvas with a strip of cardboard while rotating.

I find that sensitive organisms like *Paramoecium* live for days in culture medium containing duraluminum or aluminum filings but are killed in twenty-four hours in contact with monel metal filings. *Arbacia* eggs develop into plutei in contact with duraluminum filings.

The rate of rotation can be determined in a number of different ways, most simply by a stroboscopic method. A mark of black paint is made at one point on the rotor and this is observed with a Neon lamp [4] flashing at a rate that can be varied. This rate is obtained by setting a number of brass contact points in a bakelite commutator disk in concentric circles of say 4, 6, 8, 10, 12, 15, and 18 contacts. The disk is revolved by a variable speed motor whose speed can be read on a tachometer. A contact spring presses against the contact points on the disk, thereby applying 180 volts from B batteries to the Neon lamp. The speed of the motor is adjusted and a contact circle is selected so that the Neon lamp flashes at the same rate as the rotor revolves, when

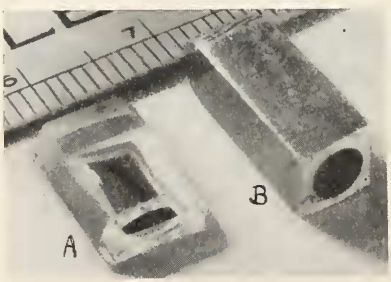

FIG. 5. Glass chamber (*A*) for microscope observation and duraluminum tube (*B*) for high speed centrifuging. The scale shows dimensions in mm.

the mark of black paint will appear to stand still. The revolutions of the motor times the number of contact points gives the speed of the rotor. It is necessary to make certain that the speed of the rotor is not some multiple of the stroboscopic rate. This can be ascertained by flashing the Neon lamp at a relatively low rate and observing the sequences of images of the black mark on the rotor as its speed increases. Three images mean ⅓ the speed of the flashing Neon lamp, two images ½ the speed, one image the same speed. When the speed of the rotor is increased to the point where a second single image appears (the Neon

[4] The Neon lamps used in television work with a large rectangular glowing electrode give enough light for use in a slightly darkened room.

lamp flashing at the same rate), its speed is twice that of the Neon lamp. In this way a curve can be plotted relating speed of the rotor to rotating pressure.

This curve can be checked in another way. The top of the rotor is painted flat black except for a small area of metal which is polished. An intense beam of parallel light is directed on the rotor so that the polished area reflects the light into a photocell. The photocell current is amplified and thrown into a loud speaker and the pitch of the sound compared with a tuning fork of known frequency. Or, an electrically driven tuning fork can be simultaneously connected with the photocell amplifier and the beats observed.

In this manner the steel rotor used for duraluminum tubes was found to have a speed of 800 R.P.S. at 20 lbs., 1,140 R.P.S. at 40 lbs., 1,460 R.P.S. at 60 lbs., and 1,800 R.P.S. at 80 lbs. rotating pressure. Since the end of the tubes are 1.5 cm. from the center of rotation the centrifugal force at 20, 40, 60, and 80 lbs. pressure is, respectively, 38,000, 78,000, 128,000, and 144,000 times gravity.

Using the tubes and egg cells suspended in isotonic sugar plus sea water mixtures of graded density, it is an easy matter in a very short

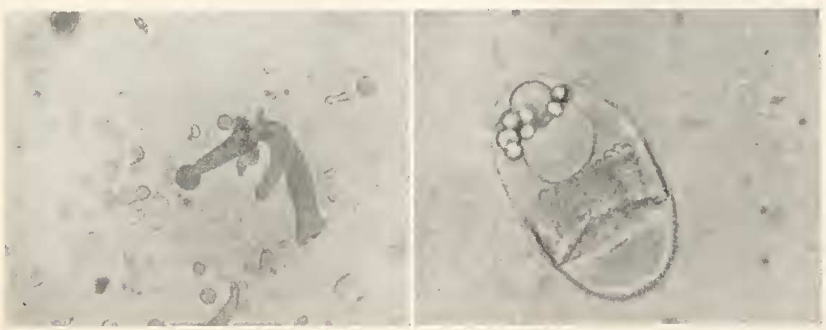

FIG. 6. (*A*) Unfertilized mature and immature starfish eggs (*Asterias forbesii*) fragmented by rotation for 10 minutes at 84,000 × g. (*B*) *Nereis limbata*, unfertilized immature eggs, rotated for 10 minutes at 84,000 × g.

time to stratify eggs which ordinarily stratify only with the greatest difficulty, such as the unfertilized egg of *Nereis* and immature and unfertilized mature eggs of the starfish. The former cannot be fragmented since the chorion is too tough, but the latter readily separate into many pieces, spheres, or elongate cylinders (Fig. 6, *A*). The cylinders slowly round up again in sea water. *Cumingia* eggs may likewise be pulled apart into small fragments. *Arenicola* and *Phascolosoma* eggs,

unfertilized, stratify readily but do not pull into fragments because of the strength of the chorion, although there is some elongation.

Nereis eggs rotated 1300 R.P.S. (84,000 × g.) for 10 minutes are somewhat elongated (Fig. 6, *B*). They develop when fertilized, producing apparently normal swimming larvae. In sea water no redistribution of the granules occurs in the unfertilized eggs even after a period of twenty-four hours.

Arbacia eggs fertilized and centrifuged for 10 minutes at 84,000 × g., 29 minutes after fertilization (at 20° C.) become markedly stratified with even the red pigment completely thrown down. With ordinary forces (10,000 × g.), many of the red pigment granules stick to the egg surface. In some eggs the oil may be pulled through the fertilization membrane. Fertilized *Arbacia* eggs subjected to such high centrifugal forces at 5, 29, 42, and 55 minutes after fertilization are all markedly stratified and many of them develop into apparently normal-looking, free-swimming blastulae. A more careful study must be made to detect possible slight abnormalities in the larvae of "supercentrifuged" eggs, since it seems almost certain that eggs which are equipotential, nevertheless contain organ-forming substances that can be moved by these forces. With non-equipotential eggs the organ-forming substances should be readily moved.

The stroma from hemolyzed erythrocytes can be readily thrown down, as well as corpuscles "reversed" from hemolysis by addition of salt. Many uses in connection with erythrocyte behavior suggest themselves.

Summary

The air turbine centrifuge, suitable for biological investigations with very high centrifugal forces, is described, together with some observations on living cells.

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